Electron spin resonance spectroscopy of oxygen radicals generated by synthetic fecapentaene-12 and reduction of fecapentaene mutagenicity to *Salmonella typhimurium* by hydroxyl radical scavenging

T.M.C.M. de Kok, J.M.S. van Maanen, J. Lankelma, F. ten Hoor and J.C.S. Kleinjans

Department of Health Risk Analysis and Toxicology and 1Department of Human Biology, University of Limburg, PO Box 616, 6200 MD Maastricht and 2Department of Oncology, Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

Fecapentaenes form a class of potent fecal mutagens and have been suggested to play an initiating role in colon carcinogenesis. Although several indications have been found that fecapentaenes may induce oxidative DNA damage as well as DNA alkylation, the mechanism of genotoxicity remains unknown. In this study, electron spin resonance spectroscopy with several spin traps has been used in order to determine whether reactive oxygen species can be formed by fecapentaene-12 (FP-12). No specific conditions could be defined that resulted in the direct formation of oxygen radicals from FP-12. However, peroxidation of FP-12 by various peroxidative enzymes has been shown to result in the formation of superoxide adducts of the spin traps α-(4-pyridyl-1-oxide)-N-t-butyl nitroxide and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). Addition of superoxide dismutase resulted in a decreased spectrum intensity, whereas the hydroxyl radical scavenger t-butyl alcohol (tBA) appeared of no influence on the signal, both confirming the formation of superoxide. The formation of hydroxyl radical spin adducts has been demonstrated after peroxidation of FP-12 in incubations with the spin-trapping agent 2,2,6,6-tetramethylpiperidine (TMP). Further, the effects of scavenging hydroxyl radicals with respect to the genotoxic potential of FP-12 in the *Salmonella* mutagenicity assay has been investigated. It was clearly shown that radical scavenging reduced the number of revertants in *Salmonella* strains TA100, TA102 and TA104. This mutagenicity-reducing effect was more convincing using both spin traps DMPO and TMP as compared to the effect of hydroxyl radical scavengers tBA and DMSO. Based on these findings, a reaction scheme is proposed that suggests the formation of superoxide after peroxidation of FP-12, which is subsequently converted to hydroxyl radicals by the iron-catalysed Haber–Weiss reaction.

**Introduction**

Fecapentaenes have been hypothesized to represent initiating agents in human colon carcinogenesis. These potent genotoxins are found in human feces (1–4) and have been suggested to originate from bacterial modification of polyunsaturated ether phospholipids (5–12). Synthetic fecapentaene-12 (FP-12*) appears to be highly mutagenic to several strains of *Salmonella typhimurium* (13–15) and various genotoxic effects have been observed in murine and mammalian cell types, comprising sister chromatid exchanges (16), mutations at the hypoxanthine-guanosine phosphoribosyl transferase locus (16), unscheduled DNA synthesis (17) and DNA single-strand breaks (18,19). However, the mechanism of fecapentaene-induced modification of DNA remains unknown. It has been proposed that fecapentaenes can form carbocations that act as alkylating agents (20), and a correlation has been found between mutagenicity and electrophilic reactivity of carbocations derived from a series of model enol ethers. Furthermore, formation of an addition product of FP-12 to a nucleophile has been described (21), as well as reduction of free thiol levels in human fibroblasts after exposure to FP-12, demonstrating the reactivity of FP-12 toward thiols (18). Preliminary studies have shown that 32P-postlabeling of DNA treated with FP-12 results in the formation of adducted nucleotides, detected as additional spots on autoradiography (22). Further, binding studies have shown that radioactivity remained associated with DNA after incubation with [3H]FP-12. It was suggested that the presumed adducts may be derived from FP-12 directly or from FP-12 decomposition fragments (22). Another mechanism that has been proposed for fecapentaene genotoxicity suggests the involvement of reactive oxygen species. High mutagenic activity has been determined in *S. typhimurium* strains TA102 and TA104, which are both relatively highly sensitive to oxidative mutagens (23). Further, *in vitro* incubation of calf thymus DNA with FP-12 or FP-14 has been shown to result in dose-dependent formation of 2'-deoxy-7,8-dihydro-8-oxoguanosine (8-oxoG) (24). Furthermore, rapid oxidation of GSH to GSSG during FP-12 exposure of human fibroblasts in *vitro* indicates that oxidative reactions may occur (18).

In this study, electron spin resonance (ESR) spectroscopy is used to detect and identify oxygen species generated by FP-12. Specific conditions that are required for this radical formation are described. Further, the influence of scavenging reactive oxygen species on the expression of fecapentaene mutagenicity to *Salmonella* is investigated. Based on these results a mechanism for fecapentaene genotoxicity is discussed.

**Materials and methods**

ESR measurements were performed at room temperature on a Bruker ESP 300 with an ESP 1600 data processor, equipped with an ER 4102 ST standard rectangular cavity. Instrumental conditions are described in the legends to the figures. α-(4-Pyridyl-1-oxide)-N-t-butyl nitroxide (POBN; Sigma) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO; Aldrich) were used for trapping both hydroxyl and superoxide radicals, whereas 2,2,6,6-tetramethylpiperidine (TMP; Aldrich) was used to trap hydroxyl radicals as well as singlet oxygen. Solutions of DMPO in nitrogen-flushed Milli-Q water were purified by gentle mixing with 30 mg/ml charcoal for 20 min at 35°C. This procedure was repeated three times to remove background ESR signals completely. The oxidized form of TMP (TEMPO), present as an impurity in the commercial product was not removed. All incubations were performed at 37°C in a total volume of 0.5 ml 0.02 M EBS, pH 7.0, containing 0.15 M KCl. Synthetic FP-12 (1.17 μmol) was incubated...
with 10 mM DMPO or 23–600 mM TMP, with or without 10 min flushing with molecular oxygen. ESR spectra were also recorded after 15 min incubation of 0.33 μmol FP-12 with 4 units of horseradish peroxidase/H₂O₂ (HRP type II, Sigma), or 200 units of prostaglandin H synthase/arachidonate (PHS; Sigma) or 200 units of lipoxidase/linooleate (LO, Sigma) and subsequent flushing with oxygen for 10 min. Since HRP requires 1–100 mM H₂O₂ as a cofactor, diethylenetri-aminepentaacetic acid (DTPA, Janssen) was added as iron chelator, to prevent spontaneous formation of OH radicals. No DTPA had to be added to incubations with PHS, since 0.2 mM arachidonate was used as cofactor. Also, incubations with LO using 0.2 mM linoleate and 1 mM H₂O₂ as cofactors could be performed without DTPA. In order to investigate the role of superoxide anion radicals in spin adduct formation, 120–500 units of superoxide dimutase (SOD; Sigma) were added. Incubation mixtures were supplemented with the OH radical scavenger t-butyl alcohol (tBA; Aldrich) (105 mM) to determine the involvement of OH radicals. Incubations were performed in deuterium oxide to discriminate between ESR signals resulting from reactions of OH radicals or singlet oxygen with TMP. Reference spectra were obtained from incubations with H₂O₂ for OH radicals, whereas the hypochlorite–H₂O₂ reaction (25) has been used to generate singlet oxygen. A nitrogen-flushed 0.5 mM solution of xanthine, supplemented with 12.5 μM adriablastina to increase superoxide production (J.M.S. Van Maanen, unpublished) was incubated with 0.05 units of xanthine oxidase (Sigma) in the presence of DTPA to form the DMPO–superoxide adduct.

Effects of oxygen radical scavenging on mutagenicity of FP-12 and of hydroxyl radical generating mutagens

Mutagenicity of synthetic FP-12 in comparison with hydroxyl radical generating mutagens was determined in S. typhimurium tester strains TA100, TA102 and TA104. A standard plate incorporation assay was used without preincubation. No metabolic activation system was added. Synthetic FP-12 was provided by A. Van der Gen (26), and stored in methanol/TEA (99.5/0.5) at −80°C (28). t-Butyl hydroperoxide (TBOOH; Sigma), cumene hydroperoxide (COOH; BDH Chemicals) and H₂O₂ (Perhydroxyl, Merck) were used as hydroxyl radical forming positive controls. Both FP-12 and control mutagens were diluted with methanol/TEA (99.5/0.5) and applied in a volume of 100 μl. Each concentration was tested in triplicate.

DMSO (Merck), tBA and DMPO, all frequently used as OH radical scavengers, were included in mutagenicity assays with control mutagens to determine their mutagenicity-inhibiting capacity in strain TA100. DMPO was dissolved in nitrogen-flushed Milli-Q water and stored under nitrogen at −20°C. In view of the chemical instability of DMPO the interval between the addition of DMPO to the test sample and onset of the mutagenicity test was kept as short as practically possible. Further, the effect of DMSO, tBA, TMP and DMPO on FP-12 mutagenicity was determined. In order to exclude interfering mutagenic or cytotoxic effects, scavengers were tested for direct mutagenicity and cytotoxicity in all three strains. Hydroxyl radical scavengers were administered in non-cytotoxic concentrations varying from 20 to 1400 μmol/plate. FP-12 concentrations of 0.5 μg/plate were used in tester strains TA100 and TA106, whereas a higher concentration of 2.5 μg/plate was necessary to induce a significant mutagenic effect in TA102. All test samples contained 50 μl methanol/TEA (99.5/0.5) with varying concentrations of FP-12 and 100 μl DMSO, tBA, TMP or DMPO solutions in Milli-Q water. Further, in Salmonella strain TA100 the effect of DMSO, tBA and DMPO on cytotoxicity of FP-12 at a toxic concentration of 2.0 μg/plate was determined. Toxicity assays were performed on histidine-enriched agar plates with 100 μg histidine per plate. Incubation mixtures were supplemented with the OH radical scavenger 1 mM DMSO (Merck), 1 mM tBA and 1 mM DMPO in triplicate.

Results

ESR spectroscopy

Incubation of synthetic FP-12 with DMPO, POBN or TMP does not result in detection of spin trap adducts. Prolonged incubation time or vigorous flushing of the incubation mixture with molecular oxygen appear to be of no influence on radical formation. Apparently, no radicals are generated by FP-12 spontaneously.

ESR spectra of incubations of FP-12 with HRP, PHS and lipoxidase (LO) are given in Figure 1, and compared with the DMPO–OOH signal obtained by generating superoxide by the xanthine/xanthine oxidase system in the presence of adriablastina. Although the hyperfine structure is not very pronounced, DMPO adducts formed after peroxidation of FP-12 resemble the DMPO–OOH spectrum (A_{N} = 14.3 G). Remarkably, DMPO–OOH spectra in these FP-12 incubations are recorded after 15 min incubation at 37°C and 10 min flushing with oxygen, whereas the DMPO–OOH signal formed by xanthine/xanthine oxidase system disintegrates completely to form DMPO–OH within 6 min. When 120 units of SOD are added to incubations with HRP and PHS, the ESR signal is decreased in intensity by respectively 64 and 31%. Since integration of ESR signals with low intensity appears not to be accurate, this reduction in intensity is calculated from signal-to-noise ratios. No effect of tBA is found on the intensity of the DMPO–OOH signal.

ESR spectra of incubations with xanthine/xanthine oxidase with and without adriablastina and of FP-12/HRP using POBN as radical trapping agent are shown in Figure 2. Splitting constants are slightly influenced by addition of adriablastina. Without adriablastina, ESR spectra of xanthine/xanthine oxidase and FP-12/HRP appear identical (A_{N} = 15.4 G, A_{D} = 2.8 G). Comparison of the spectra with the POBN–OH spectrum obtained after incubation of PBN with H₂O₂ (A_{N} = 14.8 G, A_{D} = 1.8 G) indicates that both spectra can be well distinguished. The signal intensity after incubation of FP-12 with HRP is remarkably high. This intensity is reduced by 31% upon addition of 120 units of SOD.
ESR signals of incubations of (a) xanthine/xanthine oxidase/adriablastina/DTPA/10 mM POBN, five scans after 5 min incubation, $A_N = 15.2$ G, $A_H = 2.2$ G; (b) xanthine/xanthine oxidase/DTPA/50 mM POBN without adriablastina, five scans after 5 min incubation, $A_N = 15.4$ G, $A_H = 2.8$ G; (c) FP-12/HRP/H$_2$O$_2$/DTPA/50 mM POBN, seven scans after 15 min at 37°C/10 min flushing oxygen, $A_N = 15.4$ G, $A_H = 2.8$ G. Instrumental conditions are as indicated in the legends of Figure 1.

When FP-12 is incubated with HRP/H$_2$O$_2$ in the presence of 23 mM TMP, a greatly increased signal is recorded as compared to the background TEMPO signal. However, this signal appears also to be present in reference incubations of HRP/H$_2$O$_2$ without FP-12. The intensity of this signal is not influenced by adding tBA, but is enlarged when the incubation is performed in deuterium oxide (data not shown). Apparently, HRP in combination with H$_2$O$_2$ producés singlet oxygen itself, and therefore this enzymatic system seems rather inappropriate for investigating the production of singlet oxygen by FP-12. The generation of singlet oxygen by purified peroxidase systems, including HRP, chloroperoxidase, lactoperoxidase and myeloperoxidase, has previously been demonstrated by chemiluminescence (29–32).

In Figure 3, ESR spectra of incubation of PHS with FP-12 are compared to the TEMPO signal obtained after incubation of TMP with H$_2$O$_2$. Incubation of 0.33 mM FP-12 results in a 3-fold increase of the ESR signal as compared to the control incubation without fecapentaenes (Figure 3b and c). Further, the TEMPO signal ($A_N = 17.2$ G) in FP-12 incubations is reduced to the background intensity by addition of tBA (Figure 3d), whereas it increases when FP-12 is incubated with PHS and SOD simultaneously (Figure 3e).

Effects of oxygen radical scavenging on mutagenicity of FP-12 and hydroxyl radical generating mutagens

Synthetic FP-12 appears to be mutagenic in Salmonella strains TA100, TA102 and TA104, as is shown in Figure 4. All hydroxyl radical generating mutagens induce significant numbers of revertants in these strains, indicating that these three strains are susceptible to mutagenic effects induced by hydroxyl radicals. Effects of DMSO, tBA, TMP and DMPO on the mutagenicity to strain TA104 of hydroxyl radicals originating from TBOOH or COOH are illustrated in Figure 5. Since all hydroxyl radical scavengers significantly reduce the number of revertants (except for DMSO in combination with TBOOH), this assay appears suitable to demonstrate hydroxyl radical mediated mutagenicity. Interference of radical scavenging with the mutagenicity of FP-12 is shown in Figure 6. Generally, all scavengers are shown to be capable of reducing FP-12 mutagenicity. DMPO and TMP, administered in concentrations lower by a factor of 10 and 100 respectively compared to DMSO and tBA, appear to reduce FP-12 mutagenicity most efficiently. An exception is found for TMP, which produces no significant reduction of revertants in strain TA104. Further, the mutagenicity-reducing effects of tBA are determined in strains TA102 and TA104, whereas a significant increase of the number of revertants is found in TA100. DMSO is shown to reduce FP-12 mutagenicity only in strain TA102 at a concentration of 1.4 mmol/plate. The cytotoxic effect to TA100 of 2 μg FP-12/plate is shown to be reduced by addition of hydroxyl radical scavengers (data not shown). DMPO appears to increase the number of colonies most efficiently, reaching a maximal effect at a concentration of 20 mmol/plate. Addition of DMSO and tBA results in maximal reduction of cytotoxicity.
at concentrations of 1100 and 1400 nmol/plate respectively. At the applied concentrations no mutagenic or cytotoxic effects have been shown to be induced by OH radical scavengers to any strain. However, TMP concentrations exceeding 15 μmol/plate appear to be cytotoxic to strains TA100 and TA102, and no surviving colonies of TA104 are found on plates containing 10 μmol TMP.

It is concluded that both FP-12 mutagenicity and cytotoxicity can be reduced by OH radical scavenging, and therefore OH radicals can be generated in the Salmonella mutagenicity assay in the presence of FP-12.

Discussion
Since fecapentaenes are mutagenic to Salmonella strains that are highly susceptible to oxidative damage (23) and hydroxylation of the C-8 position of guanine residues in DNA by FP-12 and FP-14 has been reported (24), it is necessary to search for reactive oxygen species produced by fecapentaenes. The formation of 8-oxodG has been shown to occur directly after in vitro incubation of calf thymus DNA with synthetic fecapentaenes (24). However, in the present study no oxygen radicals are found to be generated by FP-12 spontaneously, despite the fact that experimental conditions of both DNA incubations and ESR measurements are quite comparable; DNA incubations are performed in Tris-HCl (pH 7.4), containing 0.3 mM FP-12, at 37°C for 1–3 h, whereas ESR spectra are recorded in 0.02 mM PBS (pH 7.0), containing 0.66–2.35 mM FP-12, after 15 min at 37°C and up to 1 h scanning. During the relatively long incubation (up to 3 h) of FP-12 with DNA, oxidative DNA damage may accumulate, whereas ESR measurements have to be interrupted within 1 h due to the rapid decomposition of spin trap adducts, which in turn results in reduced signal intensity. This may provide an explanation for the inconsistency observed between these two investigations.

Enzymatic peroxidation of FP-12 by HRP, PHS or LO proves to be essential for the formation of DMPO—OOH, indicating that superoxide is generated under these conditions. The fact that SOD reduces the DMPO—OOH signals after incubation of FP-12

with HRP and PHS confirms the peroxidative formation of superoxide from FP-12. Since TBA has no effect on the signal intensity, involvement of OH radicals in DMPO adduct formation is unlikely. However, the middle two lines in the spectra of FP-12 incubations with LO and PHS (Figure 1c and d) appear slightly

Fig. 4. Mutagenicity to S.typhimurium TA100, TA102 and TA104 of: (a) synthetic FP-12; (b) t-butyl hydroperoxide; (c) cumene hydroperoxide; (d) hydrogen peroxide. *Significant mutagenicity (LSD, P < 0.01).

Fig. 5. Reduction of the mutagenic effect to S.typhimurium TA104 of: (a) t-butyl hydroperoxide (155 nmol/plate); (b) cumene hydroperoxide (107 nmol/plate), by addition of DMSO, TBA or DMPO. The concentration of DMPO is a factor 10 lower as compared to concentrations of DMSO and TBA. *Significant reduction of revertants (Student's t-test, P < 0.05).
Scheme 1. Proposed reactions occurring in vitro after peroxidation of synthetic FP-12 by various peroxidases in presence of oxygen, resulting in the generation of superoxide. OH radicals can be formed from superoxide by the iron-catalyzed Haber—Weiss reaction (B). Dismutation of superoxide by SOD results in the formation of H₂O₂ (A) which in turn may enhance the Haber—Weiss reaction and therefore OH radical formation.

More intense, and it may therefore be argued that DMPO—OH is also present. The superoxide adduct of DMPO is still present after 15 min incubation, whereas the positive control formed by the xanthine/xanthine oxidase system rapidly decays; this is probably caused by continuous production of superoxide from FP-12. Further, it has previously been described that high concentrations of superoxide destroy DMPO—OOH (33), which may explain the less pronounced hyperfine structure. In contrast to DMPO—OOH, the superoxide adduct of POBN has been shown not to decompose into POBN—OH (35). Therefore, this spin trap is used to confirm the generation of superoxide by HRP from FP-12. Addition of adriablastina to the xanthine/xanthine oxidase system rapidly decays; this is probably caused by continuous production of superoxide from FP-12. Further, it has previously been described that high concentrations of superoxide destroy DMPO—OOH (33), which may explain why disintegration of DMPO—OOH to DMPO—OH does not occur in incubations with HRP and only slightly in the presence of PHS and LO. On the other hand, it has been reported that increased formation of superoxide destabilizes the DMPO—OOH adduct (34), which may explain the less pronounced hyperfine structure. In contrast to DMPO—OOH, the superoxide adduct of POBN has been shown not to decompose into POBN—OH (35). Therefore, this spin trap is used to confirm the generation of superoxide by HRP from FP-12. Addition of adriablastina to the xanthine/xanthine oxidase system rapidly decays; this is probably caused by continuous production of superoxide from FP-12 with peroxidative enzymes. Further evidence for the production of superoxide is provided by the fact that addition of SOD reduces POBN—OOH production in incubations with HRP.

Incubation of TMP with H₂O₂ has clearly demonstrated that TEMPO is not only formed by reaction with singlet oxygen (25) but also by trapping OH radicals. Therefore, the increased signal observed after incubation of TMP with FP-12 and PHS can be attributed to the generation of either or both OH radicals or singlet oxygen. Signal reduction to the background level by addition of tBA clearly demonstrates the involvement of OH radicals. Addition of SOD to the incubation mixture results in an increased production of OH radicals. Since dismutation of superoxide generated by PHS and FP-12 produces H₂O₂ (equation A, Scheme 1), this OH radical formation is most probably caused by enhancement of the iron-catalyzed Haber—Weiss reaction by elevated concentrations of H₂O₂ (equation B, Scheme 1). This is in agreement with the observation that incubations of FP-12 with HRP, H₂O₂ and DMPO or POBN result in a decreased superoxide signal after addition of SOD, whereas no OH spin adduct is found, since in these experiments DTPA was administered to block OH radical formation via the Haber—Weiss route. It should be mentioned that the oxygen generated in the Haber—Weiss reaction might as well be in an electronically excited state (36). However, after OH radical scavenging with tBA we did not observe induction of the TEMPO signal by singlet oxygen. Since the rate constant of trapping OH radicals with TMP is unknown, it cannot be excluded that OH radicals are more efficiently trapped.

It has previously been suggested that superoxide and H₂O₂ are not likely to induce biologically relevant DNA damage themselves (37,38). Therefore, the effects of OH radical scavenging on fecapentaene mutagenicity to Salmonella have been determined. Experiments using TBOOH and COOH in strain TA104 have clearly demonstrated that mutagenicity of OH radicals can be reduced by adding OH radical scavengers. Although the mutagenic effect of FP-12 is never completely restrained,
significant reduction of mutagenicity by scavenging OH radicals demonstrates the involvement of this oxygen species. The effect is found to be most convincing using both spin traps DMPO and TMP. This more pronounced mutagenicity reduction by DMPO as compared to the effects of tBA and DMSO could be explained by the fact that DMPO traps OH radicals as well as superoxide, from which OH radicals are most probably originating. Also cytotoxicity can be diminished by radical scavenging, as determined in strain TA100, which indicates that fecapentaene-derived OH radicals are also involved in this process. Again, DMPO is found to be the most effective inhibiting compound. Since the eventually determined number of revertants integrates mutation and survival rates, the increased revertant scores induced in TA100 by tBA in the presence of FP-12 might be the result of more effective reduction of cytotoxicity.

Based on ESR measurements and the observation that mutagenicity of FP-12 can be reduced by trapping of OH radicals, we propose that enzymatic peroxidation of FP-12 occurs as indicated in Scheme 1. We show that FP-12 can be peroxidized to yield reactive oxygen species in vitro by various isolated peroxidases. No peroxidases have to be added to express fecapentaene mutagenicity in Salmonella; since fecapentaene mutagenicity can be (partially) attributed to fecapentaene-derived OH radicals, it may be concluded that OH radicals are generated by peroxidation of FP-12 in Salmonella itself. Furthermore, the specificity of peroxidase activity is shown to be of little importance for FP-12-mediated generation of superoxide in vitro, and it is therefore possible that the NAD(P)H peroxidase identified in Salmonella by Jacobson et al. (39) is involved in the intracellular activation of FP-12 during mutagenicity testing. Mutagenicity tests with addition of PHS or other peroxidases have not been conducted since exogenously generated oxygen radicals have been shown to result in cytotoxic effects rather than mutagenicity (40,41).

Carcinogenicity tests of FP-12 in rats have shown only an insignificant effect after intrarectal administration, whereas i.p. injection of FP-12 in newborn mice revealed neoplasm formation in the lung, liver, glandular stomach and development of s.c. fibrosarcoma (42,43). The results presented in this study suggest that this organ-specific carcinogenicity may result from tissue-specific peroxidase activity. PHS is known to be present in many mammalian cells and at relatively high concentrations in the lung, urinary bladder, kidney medulla and in platelets (45–47). Unfortunately, little is known about PHS concentrations in rodent colon as compared to other tissues, and no association with fecapentaene carcinogenicity in rats can therefore be made. In human colonic epithelial cells, the presence of peroxidative enzymes such as PHS (48) has been reported. As a consequence, superoxide, H$_2$O$_2$ and OH radicals may be generated from fecapentaenes after cellular resorption. Kinetic consequences of the proposed three-step reaction scheme for the expression of fecapentaene carcinogenicity imply: (i) cellular resorption of the fecapentaenes, which appears to be no problem due to their lipophilic nature; (ii) enzymatic peroxidation of fecapentaenes by PHS, resulting in efficient intracellular generation of superoxide; (iii) spontaneous or enzymatic dismutation of superoxide to form H$_2$O$_2$ and possibly singlet oxygen. It is interesting to mention that abnormalities in levels of cellular SOD have been reported in colon tumors (49,50); site-specific generation of OH radicals near the DNA. Catalysis of this reaction by chromatin-bound iron has been proposed to play a key role in H$_2$O$_2$/superoxide-induced DNA damage (51). Apart from a direct interaction with the genetic material, reactive oxygen species may interact with DNA repair/replication processes and induce nucleotide pool imbalances that indirectly cause genetic damage (52). In conclusion, it can be hypothesized that exposure to fecapentaenes may result in oxidative DNA damage in vivo, and whether this damage is related to carcinogenic events. The toxicokinetic properties and the stability of fecapentaenes under physiological conditions will have to be investigated in order to determine the possible risk for fecapentaene-induced carcinogenesis in other tissues high in peroxidative activity, like the lung, bladder and prostate (48).

References


Received on September 25, 1991; revised on March 18, 1992; accepted on April 1, 1992

ESR of oxygen radicals generated by FP-12